

AMENDMENTS TO THE CLAIMS:

The following is the status of the claims of the above-captioned application, as amended.

Claims 1-49 (Canceled).

Claim 50 (Previously presented). A bacterial host cell comprising at least two amplified copies of an amplification unit, wherein the amplification unit is integrated in the genome of the bacterial host cell and the amplification unit comprises:

a) at least one copy of a gene of interest, and

b) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having an activity lower than the endogenous promoter of the conditionally essential gene, wherein the conditionally essential gene encodes an enzyme from the biosynthetic pathway of an amino acid, and wherein the conditionally essential gene if not functional would render the cell auxotrophic for the amino acid.

Claim 51 (Canceled).

Claim 52 (Currently amended). The cell of claim 5150, wherein the bacterial prokaryotic host cell is a gram-positive cell.

Claim 53 (Currently amended). The cell of claim 5250, wherein the bacterial gram-positive host cell is a species of the genus *Bacillus*.

Claim 54 (Previously presented). The cell of claim 50, wherein the gene of interest encodes an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, carbohydrolase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, or xylanase.

Claim 55 (Previously presented). The cell of claim 50, wherein the gene of interest encodes an antimicrobial peptide.

Claim 56 (Previously presented). The cell of claim 50, wherein the gene of interest encodes a peptide with biological activity in the human body.

Claim 57 (Previously presented). The cell of claim 50, wherein the conditionally essential gene encodes one or more polypeptide(s) involved in lysine, leucine or methionine synthesis.

Claim 58 (Canceled).

Claim 59 (Previously presented). The cell of claim 58, wherein the conditionally essential gene is at least 90% identical to the *metE* sequence of *Bacillus subtilis* shown in positions 997 to 2199 of SEQ ID NO: 16.

Claim 60 (Previously presented). The cell of claim 59, wherein the conditionally essential gene is at least 95% identical to the *metE* sequence of *Bacillus subtilis* shown in positions 997 to 2199 of SEQ ID NO: 16.

Claim 61 (Withdrawn). The cell of claim 50, wherein the amplification unit further comprises an antibiotic selection marker, preferably the selection marker is flanked by resolvase sites or *res*-sites.

Claim 62 (Withdrawn). The cell of claim 50, wherein the amplification unit further comprises a resolvase site or *res*-site.

Claim 63 (Withdrawn). The cell of claim 50, wherein the conditionally essential gene comprised in the amplification unit has at least one transcription terminator located upstream of the gene.

Claim 64 (Previously presented). The cell of claim 50, wherein the conditionally essential gene is transcribed from a heterologous promoter having an activity level, when compared with the endogenous promoter of the conditionally essential gene, which is reduced by a factor of 2 to 100.

Claim 65 (Previously presented). The cell of claim 50, wherein the conditionally essential gene is promoterless.

Claim 66 (Previously presented). The cell of claim 65, wherein the gene of interest is located upstream of the conditionally essential gene in the amplification unit, and wherein the two genes are co-directionally transcribed.

Claim 67 (Previously presented). The cell of claim 66, wherein the conditionally essential gene is expressed by read-through transcription from the gene of interest.

Claim 68 (Withdrawn). A method for producing a protein encoded by a gene of interest, comprising

- a) culturing a bacterial host cell of claim 50; and
- b) recovering the protein.

Claim 69 (Withdrawn). A method for producing a protein encoded by a gene of interest, comprising

- a) culturing a bacterial host cell comprising at least two duplicated copies of an amplification unit in its genome, the amplification unit comprising:
 - i) at least one copy of the gene of interest, and
 - ii) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having an activity lower than the endogenous promoter of the conditionally essential gene, and wherein the conditionally essential gene encodes an enzyme from the biosynthetic pathway of an amino acid,
- wherein the conditionally essential gene if not functional would render the cell auxotrophic for the amino acid; and
- b) recovering the protein.

Claim 70 (Withdrawn). A method for producing a bacterial cell comprising two or more amplified chromosomal copies of a gene of interest, the method comprising:

- a) providing a bacterial cell comprising at least one copy of an amplification unit, the unit comprising:
 - i) at least one copy of the gene of interest, and
 - ii) an expressible functional copy of a conditionally essential gene, which is either promoterless or transcribed from a heterologous promoter having an activity lower than the endogenous promoter of the conditionally essential gene, and wherein the conditionally essential gene encodes an enzyme from the biosynthetic pathway of an amino acid,

wherein the conditionally essential gene if not functional would render the cell auxotrophic for the amino acid;

- b) cultivating the cell under conditions suitable for growth in a medium deficient of the amino acid, thereby providing a growth advantage to a cell in which the amplification unit has been duplicated in the chromosome; and
- c) selecting a cell wherein the amplification unit has been duplicated in the chromosome, whereby two or more amplified chromosomal copies of the gene of interest were produced.

Claim 71. (Previously presented) A bacterial host cell comprising at least two copies of an amplification unit, wherein the amplification unit is integrated in the genome of the bacterial host cell and the amplification unit comprises:

- a) at least one copy of a gene of interest, and
- b) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having an activity lower than the endogenous promoter of the conditionally essential gene, wherein the conditionally essential gene encodes an enzyme from the biosynthetic pathway of an amino acid, wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance, wherein the gene of interest is located upstream of the conditionally essential gene in the amplification unit, and wherein the two genes are co-directionally transcribed.

Claim 72 (Previously presented). The cell of claim 50, wherein the conditionally essential gene is at least 98% identical to the *metE* sequence of *Bacillus subtilis* shown in positions 997 to 2199 of SEQ ID NO: 16.